PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 59.68.75481/002	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/00195	International filing date *(day/month/year)* 15.01.2003	Priority date *(day/month/year)* 15.01.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant MATFORSK et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15.08.2003	25.06.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Botz, J Telephone No. +31 70 340-4513

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/00195

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-45 as originally filed

Claims, Numbers

1-26 as originally filed

Drawings, Sheets

1/20-20/20 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 17-20,25

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 17-20,25 are so unclear that no meaningful opinion could be formed *(specify)*:

   see separate sheet

   ☒ the claims, or said claims Nos. 17-20,25 are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	11,23
	No:	Claims	1-10, 12-16, 21, 22, 24, 26
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-16,21-24,26
Industrial applicability (IA)	Yes:	Claims	1-16,21-24,26
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Present **claims 17 - 20** and **25** relate to an extremely large number of possible compounds. In fact, the claims contain so many options and variables that a lack of clarity and conciseness within the meaning of **Article 6 PCT** arises to such an extent as to render a meaningful examination of the claims impossible. In addition claims 17 - 20 and 25 are defined as an effect to be achieved and therefore as desideratum (" (sequence) regions which are not naturally defined in juxtaposition" / "either side of a junction region between a regulatory region and a coding region within sample nucleic acid". No opinion during the examination phase will therefore be provided on claims 17 - 20 and 25 with regard to novelty, inventive step and industrial applicability.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2.1 **CITATIONS**

Reference is made to the following documents:

D1: WO 00 75369 A (REPP REINALD ;RASCHER WOLFGANG (DE)) 14 December 2000 (2000-12-14)

D2: BROWNIE JANNINE ET AL: 'The elimination of primer-dimer accumulation in PCR' NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 25, no. 16, 1997, pages 3235-3241, XP002152588 ISSN: 0305-1048

D3: WO 01/55454 A (GENETRACE SYSTEMS INC ;LOEHRLEIN CHRISTINE (US); MONFORTE JOSEPH A) 2 August 2001 (2001-08-02)

D4: POLZ M F ET AL: 'Bias in template-to-product ratios in multitemplate PCR.' APPLIED AND ENVIRONMENTAL MICROBIOLOGY. UNITED STATES OCT 1998, vol. 64, no. 10, October 1998 (1998-10), pages 3724-3730, XP002261301 ISSN: 0099-2240

D5: GRACE MARCY B ET AL: "Degradable dUMP outer primers in merged tandem (M/T)-nested PCR: Low- and single-copy DNA target amplification" ANALYTICAL BIOCHEMISTRY, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 263, no. 1, 1 October 1998 (1998-10-01), pages 85-92, XP002152587 ISSN: 0003-2697

D6: HEATH K E ET AL: 'Universal primer quantitative fluorescent multiplex (UPQFM) PCR: a method to detect major and minor rearrangements of the low density lipoprotein receptor gene.' JOURNAL OF MEDICAL GENETICS. ENGLAND APR 2000, vol. 37, no. 4, April 2000 (2000-04), pages 272-280, XP001055883 ISSN: 0022-2593 cited in the application

2.2 NOVELTY (Art. 33(2) PCT)

2.2.1 **D1** discloses a quantitative multiplex PCR technique applying in the first PCR reaction primers that introduce probe binding sites to enable the use of the same primer sequence for different targets. The primers contain complementary tail regions (homo-tags) to prevent primer-dimerisation products as well as uracil, which enables their digestion by the enzyme Uracil-DNA-glycosylase before the second primers are used, c.f. pages 1 - 16 and claims 1 - 27. It is to be pointed out, that claim 1 demands the primers to comprise a bipartite structure with one part being specific for a particular target sequence, and the other part, being a constant sequence, common to all primers or common amongst all forward primers. The IPEA is of the opinion that this is the case here, c.f. example for page 28 / Interleukin primers. Part B, the constant part, consists of a common sequence, composed of the sequence printed in italic and the one in bold letters. The sequence underlined is the gene-specific sequence (part A). It is to be pointed out here, that claim 1 defines "bipartite structure" as a primer being composed of a genespecific part, represented in **D1** by the underlined sequence, and a *constant* sequence "which is common ... amongst all forward primers, with a different sequence common amongst all reverse primers". Such a formulation does not exclude the constant part of the sequence to be composed of two functional sequences, c.f. sequence identities 11, 12 and 13. The subsequent PCR-reactions are designed in such a way, that a first PCR-reaction takes place with the bipartite primer, said bipartite primer being composed of Uracil- instead of Thymidin-bases within the gene-specific part, thereby allowing the digestion of said gene-specific part with the enzyme uracil-N-glycosylase, care for page 9, first paragraph. The first PCR reaction is performed for only 3 - 5 cycles, thereby encompassing the feature of dependent claim 4. The second PCR-reaction is then performed via part B of said bipartite primer, the so called "Homo-Tails vermittelte" amplification", c.f. page 9, lines 5 to 25. In this respect, prior art **D1** mentions as underlying principle ("das zugrundeliegende Prinzip") of the application the so called "Homo-Tag-Assisted-Non-Dimer-System", c.f. page 8, lines 14 to 19, the latter being outlined

in Figure 1 of prior art **D3**. The applicant is correct in mentioning, that the PIRS probe "does not participate in a further amplification step". This amplficiation step however is performed exclusively via the homo-tail ***before*** said probe is applied. In summary, although prior art **D1** comprises a detection mechanism not embodied by the underlying application, namely the "addition of a probe" as formulated by the applicant in the letter of April 23rd 2004, steps (a) to (e) of the method of the underlying application are nevertheless comprised in **D1**.

2.2.2 Prior art **D1** is novelty-destroying to **claims 1 - 10, 12 - 16, 21, 22, 24** and **26**.

2.3 INVENTIVE STEP (Art. 33(3) PCT)

2.3.1 In case, the applicant should overcome the above mentioned novelty-objections, the following reasoning with respect to inventive step (**Art. 33(3) PCT**) would be pursued:

2.3.2 Document **D3** is considered to be **the closest prior art** for **claims 1 - 16, 21 - 24** and **26** and comprises a technique for a quantitative multiplex PCR. The primers have a bipartite structure, with a first sequence derived from a target gene of interest and positioned within the 3' region of the oligonucleotide, and a second sequence that is complementary to a universal primer and positioned within the 5' region of the oligonucleotide. As such, a universal primer does not hybridize to the target sequence template during a PCR reaction, c.f. pages 2 - 18. Primers are designed such, that the annealing temperature of the unversal sequence is higher / greater than that of the target-specific sequences. The method employing these primers further include increasing the annealing temperature of the reaction after the first few rounds of amplification. This increase in reaction temperature suppresses further amplification of sample nucleic acids by the target specific primers, and drives the amplification by the universal primers. By choosing more stringent annealing temperatures for the second amplification reaction, artefacts like primer-dimer are avoided.

2.3.3 The **difference** of the underlying application with respect to the closest prior art is, that in order to enhance the quantitative character of the method, the bipartite primers of the first PCR reaction are physically removed, by, e.g. digestion, whereas in **D3** a higher annealing temperature is chosen for the second PCR reaction, both means helping to avoid artefacts such as primer-dimers.

2.3.4 The **problem** of the underlying application is the provision of a ***quantitative character*** for the multiplex PCR.

2.3.5 The **solution** to this problem is according to the underlying application the removal of the first (bipartite) primer-species prior to the amplification with the second, gene-specific primer-species. This is achieved by degrading or separating the bipartite primers from the amplification products of the first amplification reaction, prior to performing the second amplification reaction.

2.3.6 This solution however cannot be considered as meeting the criteria of **Article 33(1)** and **(3) PCT**:

2.3.7 The removal / digestion of the first primer species prior to the amplification with the second, gene-specific primer-species is considered as a means to enhance the quantitative character of the method thereby avoiding the generation of artefacts, such as primer-dimers. The application of a higher annealing temperature chosen for prior art **D3** is considered as an alternative embodiment serving a similar purpose.

2.3.8 The removal / digestion of the first primer species prior to the amplification with a second primer species is though known from prior art, **D5**. Here, the removal / digestion of the first primer species is applied within a nested PCR technique to remove the first of two subsequently used primer-species, c.f. the whole publication and in particular for the paragraph "Uracil N-Glycosylase Degradation Reactions" on page 87.

2.3.9 The skilled person would therefore regard it as a normal option to include said embodiment, namely the removal / digestion of the first primer species prior to the amplification with a second primer species, in order to solve the problem posed.

2.3.10 The removal / digestion of the first primer species prior to the amplification with a second primer species is therefore merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed.

2.3.11 The present application does not meet the criteria of **Article 33(1) PCT**, because the subject-matter of **claims 1 - 16, 21 - 24** and **26** does not involve an inventive step in the sense of **Article 33(3) PCT**.